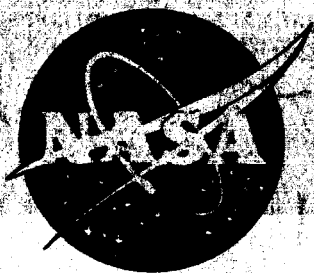


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THE SQUIRREL MONKEY (SAIMIRI SCIUREUS)

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Research Report

HISTOPATHOLOGIC EVALUATION OF A LABORATORY PRIMATE:

THE SQUIRREL MONKEY (SAIMIRI SCIUREUS)*

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Bureau of Medicine and Surgery
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U. S. NAVAL SCHOOL OF AVIATION MEDICINE
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SUMMARY PAGE

THE PROBLEM

Numerous physiological and psychological stress studies oriented toward the evaluation of primates as the biological indicators of the effects of hostile environments are presently being conducted. Primary emphasis of these studies is the preparation for and the eventual placement of primates in orbiting satellites for prolonged periods of time.

Since the squirrel monkey is being utilized as the biological model in a variety of experiments, a requirement has been established to record baseline data on these small subhuman primates. The major emphasis of the present study was to establish an impression of normal microanatomy of the major organ systems.

FINDINGS

Post-mortem examination of forty-five squirrel monkeys was conducted. Tissues from the major organ systems of fifteen of these animals were prepared for microscopic evaluation. Eleven of these monkeys, initially classified as essentially normal animals, were found to have numerous alterations of tissue structure, reflecting various types of inflammatory and degenerative lesions. In addition, histopathologic evaluation of the remaining four animals was conducted to further establish and clarify the gross findings at necropsy of pulmonary disease and helminthiasis of the gastrointestinal tract.

RECOMMENDATIONS

The attention of investigators utilizing the squirrel monkey as an experimental animal is directed to the possible existence of acute and chronic lesions in apparently normal animals. Interpretation and evaluation of subtle alterations in function or structure of the animal being used as a biological model must be meticulously conducted, and careful histopathological evaluation of major organ systems is an essential requirement for the proper interpretation of a cause-effect relationship.

ACKNOWLEDGMENTS

The author gratefully acknowledges the technical assistance of Mr. C. S. Ezell and Mr. C. C. Crawford.

INTRODUCTION

In recent years there has been an increase in interest and accumulation of knowledge regarding the squirrel monkey, Saimiri sciureus. Considerable attention is now being given to the use of this small primate as a promising experimental animal in a variety of biological research programs. During the past year several environmental stress studies were conducted in which the squirrel monkey served as the biological model. While the use of other more common laboratory animals such as the mouse and rat have proven satisfactory for a variety of investigations of compound environmental stresses, currently a subhuman primate, the squirrel monkey, is being evaluated in this laboratory as a possible subject in an investigation of the effects of decompression, hyperoxia, and radiation. Additionally, this primate is being utilized in programs concerned with vestibular physiology and also with the biological effects of magnetic fields on the intact animal.

Concurrent with these studies was an attempt to establish a gross and microscopic impression of the normal anatomical structures of this primate. Interpretation of the morphological alterations produced in the monkeys being subjected to an experimental stress requires a basic knowledge of any subclinical pathology which may be present in the animal and which, if undetected, could lead to a false interpretation by the investigator as to the possible effect of an experimental condition.

In spite of the numerous physiological and psychological investigations carried out over the past few years, scant information is available concerning clinical and sub-clinical pathology of the squirrel monkey, as noted in a recent report (1). It is the purpose of the present report to record some of the more common histopathological findings in a selected group of squirrel monkeys that had been kept in a laboratory colony rather than in their natural habitat.

PROCEDURE

Animals utilized in this study were forty-five adult squirrel monkeys, of both sexes, weighing between 450 and 700 grams, taken from a laboratory colony maintained in accordance with the methods previously described by Beischer and Furry (2). Of the total number of animals submitted for post-mortem examination, thirty were not selected for further extensive histological evaluation because of their participation in other experimental programs. The remaining fifteen were divided into two groups: Eleven were classified as "normal" and given a code number with the prefix N; four were placed in code group P because of the fact that there was clinical evidence of disease prior to death.

Monkeys in group N had been in the laboratory animal colony at least one year prior to necropsy. They were sacrificed with an intraperitoneal injection of Sodium Pentobarbital (300 mg), and autopsy procedures were begun after adequate evidence of absence of vital signs. The animals in group P were examined within one hour after death. In all cases the examination followed the development of an acute clinical episode characterized by the abrupt onset of anorexia followed by coma which terminated with the cessation of vital functions.

To demonstrate microscopic anatomy, representative blocks of tissue from the heart, ascending aorta, lungs, kidneys, adrenals, ovaries, testes, uterus, salivary glands, esophagus, trachea, stomach, small intestine, colon, and brain were fixed in buffered neutral formalin. Selected tissue blocks were then washed, dehydrated, embedded in paraffin, and sectioned at 10 microns. Ten to twenty-five serial sections were prepared from each block; at least five of these sections were stained with hematoxylin and eosin. A variety of special stains were used on some sections for various connective tissue elements and to demonstrate special cellular morphology.

RESULTS

GROUP N

The most significant findings in this present study were the numerous lesions observed during the review of the histological sections of the eleven apparently normal animals in group N (Table I).^{*} The most frequent lesions found were a reflection of both acute and chronic cellular infiltration of both the peribronchial and perivascular areas of the lungs. Additionally, it was not uncommon to find large segments of the lung in various stages of resolution from pneumonia of unspecified causes (Figure 1). In one lung a very pronounced parasitic infestation could be demonstrated along the lateral margins of the pulmonary parenchyma (Figure 2). Numerous giant cells, dense areas of lymphocytes, and areas of proliferation of the alveolar epithelium were occasionally observed (Figure 3).

In most of these animals various renal diseases were found which included large areas of fibrosis extending through both the cortex and medulla (Figure 4). There was adequate evidence of both acute and chronic glomerulonephritis. Renal interstitial inflammatory cell infiltrates extending throughout the glandular portion of the kidney were typical of acute pyelonephritis (Figure 5).

The liver was frequently the site of well-organized foci of lymphocytic cellular infiltrates (Figure 6), and it was not uncommon to see areas of portal necrosis. A dense lymphocytic infiltration was noted in the lamina propria of some of the larger pancreatic ducts of one animal. One lesion was detected in an isolated area of the ascending aorta

^{*}In order not to break the continuity of the text, all tables and figures appear at the end of the report.

which was characterized by endothelial proliferation with hemosiderin and a well-defined, dense, eosinophilic area in the adjacent intima (Figure 7). A well-organized renal vein thrombus (Figure 8) was detected in tissue sectioned to show the relationship of both kidneys and both adrenals, including the adjacent vascular structures.

The stroma of both ovaries of one monkey was so densely infiltrated with lymphocytes that only occasional areas of germinal epithelium could be detected. Multiple foci of adipose tissue in a spleen caused it to appear in the low power field as if it were "moth eaten." An isolated area of cortical degeneration was observed in the cortex of the adrenal gland. The remnants of the nuclei were densely staining and pyknotic, and the surrounding area had lost all evidence of cellularity. A minimal inflammatory cell response was noted at the margins of this well-defined island of degenerated tissue. An extensive aggregation of lymphocytes was observed in the medulla of yet another adrenal gland; this cellular infiltration occupied most of the medullary area.

GROUP P

Histopathologic evaluation of the four animals in group P that had died during an abrupt clinical episode revealed numerous lesions in the pulmonary tissues (Table II). These ranged from moderate to extensive perivascular and peribronchial cellular infiltrates, indicating both acute and chronic inflammatory cell responses, to almost complete obliteration of the normal stromal architecture with extensive necrosis and patchy areas of atelectasis (Figure 9).

Helminthic infestation of the gastrointestinal tract was demonstrated in sections of both the small and large intestines in all four monkeys. In one animal numerous parasites in various degrees of penetration of the enteric coats were seen in the colon. There was extensive granuloma formation in the submucosa and adequate histological evidence that the organism had penetrated the serosal layer (Figure 10). This confirmed the gross finding of peritonitis. Also in this animal there was positive evidence of necrosis on the periphery of the spleen which was observed at autopsy as a typical "sugar coated" spleen. Also observed in this animal microscopically was evidence of extensive necrosis of the liver and pyelonephritis.

GENERAL OBSERVATIONS

While the primary purpose of this study was to evaluate the microscopic anatomy of the major organ systems, observations were made during the initial post-mortem examination of the remaining thirty monkeys which seem worthy of note. Since a moderate amount of gross pathology was in evidence, it would seem expedient to include in the findings a general discussion of the more essential, and in some instances unexpected, alterations of normal structure. While parasitic infestation of the gastrointestinal tract was all too apparent in the majority of animals, one had a perforation of the colon, with liberation of fecal material into the peritoneal cavity. The entire colon was dark red to

purple in color, and there was evidence of complete prolapse of the rectum with associated rectal bleeding. The small intestine was dark red in color along the course of the inferior half of the ileum, while the superior segments were not involved in this process; no evidence of mechanical obstruction could be demonstrated at necropsy. A surgically induced perforation of the esophagus was detected in an animal that died in acute pulmonary congestion during the second post-operative day following a bilateral vagotomy just below the level of the bifurcation of the common carotid arteries. An animal with moderate clinical ataxia was found at necropsy to have residual evidence of an extensive subdural hematoma. Three animals died as a result of apparent uncontrolled uterine hemorrhage within six hours following spontaneous incomplete abortions.

Inasmuch as the clinical histories of the animals studied were varied, fragmentary, or in some instances lacking, no significant clinical-pathological correlation is justified. The general impression gained from a review of the initial autopsy examination records was that, even in animals maintained in the laboratory animal colony for over one year, there is a moderate amount of helminthic infestation despite the fact that all of the animals had been given some form of antihelminthic therapy during the time they were in the colony. Macroscopic evidence of pulmonary disease was also seen in approximately one half of the animals examined.

DISCUSSION

While the major emphasis in the present investigation was to establish an impression of normal microanatomy, it soon became apparent that, from the study of tissues of a group of monkeys initially selected as "normal," prepared originally to serve as representative of normal material, numerous and varied lesions of an inflammatory and degenerative nature would be encountered. Although it was surmised that many of these lesions were a reflection of multiple infectious processes of either an acute or chronic nature, viral, bacterial, or parasitic, only exceptionally was demonstration of the organism possible. Thus there remains a requirement for extensive clinical evaluation of these primates to discern the prevalence of bacterial and parasitic organisms which may find the squirrel monkey an adequate host despite the rather benign environment and the controlled conditions of the laboratory colony in which it is now maintained.

Although it is not possible at present to relate the essential etiologic agent to the observed alteration of normal structure, it is quite obvious that numerous subclinical lesions exist in apparently normal primates. [For a discussion of comparative pathology in various primates see Lapin and Yakovleva (3) and Utkin (4).] Taken as a group, these lesions would indicate a progressive mechanism of deterioration of cellular and intracellular elements. These, along with a concomitant decline in tissue function, eventually lead to a clinically recognizable disease entity which if virulent enough may terminate with the demise of the organism. While the manifestation of disease may be due to the interaction of a number of environmental variables, it is obvious that certain stress

experiments may well require functional tissue reserves to be expended which some time previously had been altered or even completely destroyed by some insidious disease processes.

Obviously, any report of mortality is inappropriate since while at the present time our primate colony may have upwards of 150 animals as residents, they are for the most part in a "transient" status, and very few reside for periods of over one year without being assigned to an investigator. It was fortunate indeed that eleven of these animals were available for study at this time. Although it is not only time consuming but extremely costly to maintain such a study population, there is definite merit in a temporal study of mortality and morbidity in a laboratory-maintained colony of these primates.

The pulmonary pathology noted in this present study should be especially kept in mind when using the animal in cardiovascular or pulmonary function oriented research programs. The pulmonary, renal, and liver lesions were present in these animals for a considerable period of time prior to their being selected for histological evaluation, as evidenced by the chronic nature of the cellular and intracellular morphological changes observed in the sections studied. Some investigators would tend to dismiss these lesions if they appeared also with the same relative frequency in the control population, while investigators with limited experience in necropsy may fail to note the presence of any gross alteration, and subsequently fail to obtain adequate histopathologic studies.

It is essential and a primary requirement for investigators utilizing this animal, therefore, to be aware of the need for basic data concerning the mortality and morbidity, including some expression of types of diseases, both clinical and subclinical, in a laboratory population of these primates. This also should include physiological and biochemical data collected as part of either an experimental or control study. Acute stress experiments on animals with pre-existing chronic infectious diseases can severely alter survival curves, although relatively minimal tissue injury was incurred as a result of the actual experiment. Some lesions regarded as induced by the experiment may indeed be reflections of the exacerbation of a pre-existing disease. In all cases gross examination of both experimental and control animals should be followed by histological evaluation in order to rule out insidious deterioration in the tissues not related to the experiment per se.

CONCLUSIONS

The attention of investigators utilizing the squirrel monkey as an experimental animal is directed to the possible existence of acute and chronic lesions in apparently normal animals. Interpretation and evaluation of subtle alterations in function or structure of the animal being used as a biological model must be meticulously conducted, and careful histopathological evaluation of major organ systems is an essential requirement for the proper interpretation of a cause-effect relationship.

Such frequent observations as substantial leukocytic infiltrations of the bronchial and vascular structures of the lung and the demonstration of parasitic organisms in the alveolar areas should in themselves call for a more detailed appreciation of altered pulmonary function in these primates. Nutritional or toxicity studies involving evaluation of liver cellular function or structure should also be cautiously and carefully reviewed to rule out the interaction of pre-existing disease. Most significant is the finding of acute and chronic renal lesions with a variety of inflammatory and degenerative tissue changes. Alterations in renal function may mirror not only systemic variations in cardiovascular dynamics but also focal renal pathology. The ever present threat of helminthiasis as a factor in the alteration of the expected physiological response in these animals is not to be dismissed as a minor or unrelated problem. In the final analysis, a concerted attempt should be made to establish a laboratory colony population of these primates which have been brought to a level of health comparable with other laboratory animals.

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Table 1

Essential Microscopic Evaluation of Eleven Apparently Normal Squirrel Monkeys

Animal Number	System			
	Respiratory	Vascular	Digestive	Urogenital
N-1	Lung: Aggregates of lymphoid tissue adjacent to the bronchial epithelium; hyperplasia of bronchial mucosa; lymphocytic infiltration of stroma.	Spleen: Giant cells in germinal centers.	Pancreas: Lymphocytic infiltration in lamina propria of large ducts.	Kidney: Acute inflammatory cell infiltrate in medulla and cortex; moderate arteriolar fibrosis.
N-2	Lung: Increased density of stromal elements with moderate peribronchial lymphocytic infiltration.	Coronary arteries: Perivascular lymphocytic infiltration.		Ectocervix: Area of acute inflammatory cells in epithelium with early fibrosis invading stratified squamous epithelium. Kidney: Hypercellularity of glomeruli.
N-3	Lung: Minimal peribronchial lymphocytic infiltration; increased stromal thickening.	Spleen: Multiple foci of adipose cells.	Stomach: Lymphocytic infiltration of lamina propria.	Kidney: Moderate fibrosis of some glomeruli and arterioles.
N-4	Lung: Numerous areas of consolidation with lymphocytes, giant cells and early fibrosis; peribronchial lymphocytic infiltration.			Adrenal: Foci of acute inflammatory cells in cortex, adjacent to capsule.
				Kidney: Glomerular degeneration; foci of acute inflammatory cells in cortex, and interstitial hemorrhage in medulla.
N-5	Lung: Multiple foci of leukocytes, and cellular debris in alveoli.	Renal vein: Organized thrombus.	Small intestine: Helminthiasis; metaplasia of mucosal epithelium; chronic inflammatory cells in mucosa and submucosa. Liver: Foci of leukocytic infiltration.	Adrenal: Aggregate of lymphoid tissue in medulla.
				Adrenal: Area of nuclear pyknosis and loss of cellular structure in cortex.

N-6	<u>Lung:</u> Areas of fibrosis with giant cells and lymphocytes.		<u>Kidney:</u> Extensive fibrosis of glomeruli.	
N-7	<u>Lung:</u> Extensive areas of consolidation with giant cells; interstitial fibrosis. Peribronchial lymphocytic infiltration.		<u>Kidney:</u> Hypercellularity with leukocytic cell infiltration; degenerating tubules, and glomeruli. <u>Ovary:</u> Extensive lymphocytic invasion of germinal epithelium and stroma.	
N-8	<u>Lung:</u> Helminthiasis in lateral margins of alveoli with stromal cell infiltrate extensive peribronchial lymphocytic infiltration; giant cells, and fibrin. <u>Cardiac muscle:</u> Small areas of interstitial hemorrhage. <u>Aorta:</u> Endothelial proliferation with intimal thickening.		<u>Kidney:</u> Extensive fibrosis involving both cortex and medulla with fibrosis of blood vessels; lymphocytic infiltration of interstitial tissue with obliteration of tubules and glomeruli.	
N-9	<u>Lung:</u> Extensive areas of consolidation with chronic inflammatory cells, and giant cells; peribroncholar lymphocytic infiltration.		<u>Kidney:</u> Hypercellularity of glomeruli; thickening of arterioles; some obliteration of glomerular elements by lymphocytic infiltrates.	
N-10	<u>Lung:</u> Extensive interstitial fibrosis; small foci of chronic inflammatory cells. <u>Spleen:</u> Endothelial proliferation and thickening of arterioles.	<u>Liver:</u> Well-defined areas of parenchymal nuclear pyknosis and cellular debris.	<u>Ovary:</u> Extensive lymphocytic infiltration of stroma with obliteration of most germinal epithelium.	
N-11	<u>Lung:</u> Moderate stromal proliferation; pronounced perivascular and peribronchiolar lymphocytic infiltration.	<u>Liver:</u> Small foci of lymphocytic infiltration and peri-portal lymphocytic infiltration.	<u>Kidney:</u> Scattered foci of acute inflammatory cells in cortex, minimal glomerular degeneration, and thickening of arterioles.	

Table II

Essential Microscopic Evaluation of Four Squirrel Monkeys with Prior Evidence of Disease

Animal Number	System			Urogenital
	Respiratory	Vascular	Digestive	
P-1	Lung: Hyperplasia of Bronchial mucosa; peribronchial lymphocytic infiltration.		Small intestines: Helminthiasis of small intestines.	
P-2	Lung: Moderate diffuse interstitial fibrosis.		Liver: Foci of necrosis; moderate fibrosis. Colon: Helminthiasis of the colon.	
P-3	Lung: Perivascular lymphocytic infiltrate; interstitial fibrosis.		Small intestine: Helminthiasis of the small intestine. Liver: Bacilli in large veins of liver.	Testes: Acute inflammatory cell infiltrate in tunica of testes.
P-4	Lung: Acute inflammatory cells; remnants of alveolar cells and reticulum in islands of cellular debris and fibrin.	Spleen: Acute inflammatory cells; nuclear debris along periphery.	Large intestine: Helminthiasis with perforation and extensive areas of granuloma. Liver: Extensive areas of parenchymal necrosis.	Kidney: Glomerular fibrosis; foci of necrosis in cortex.

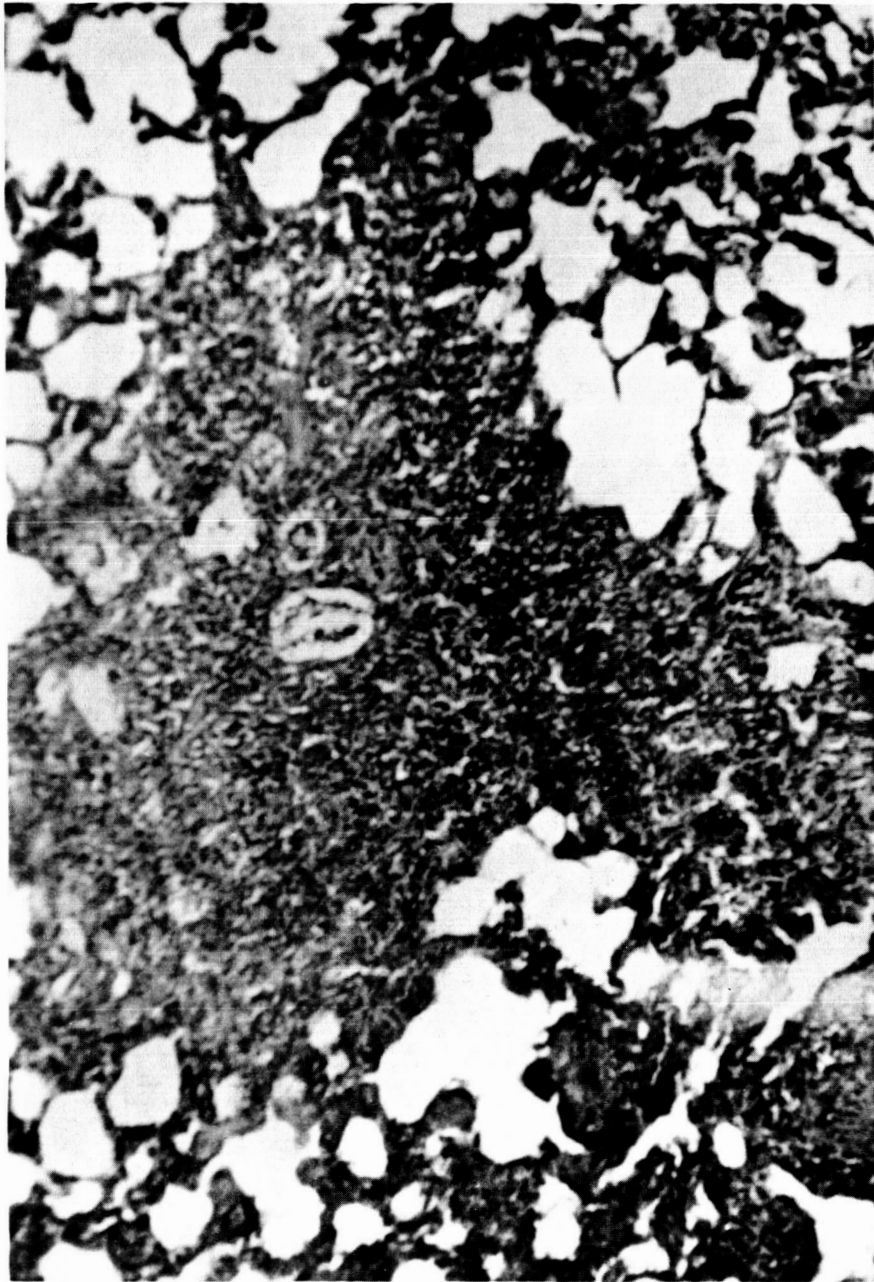


Figure 1

Lung of Squirrel Monkey N-7
Area of consolidation with remnants of a bronchiole.
H and E. X 100

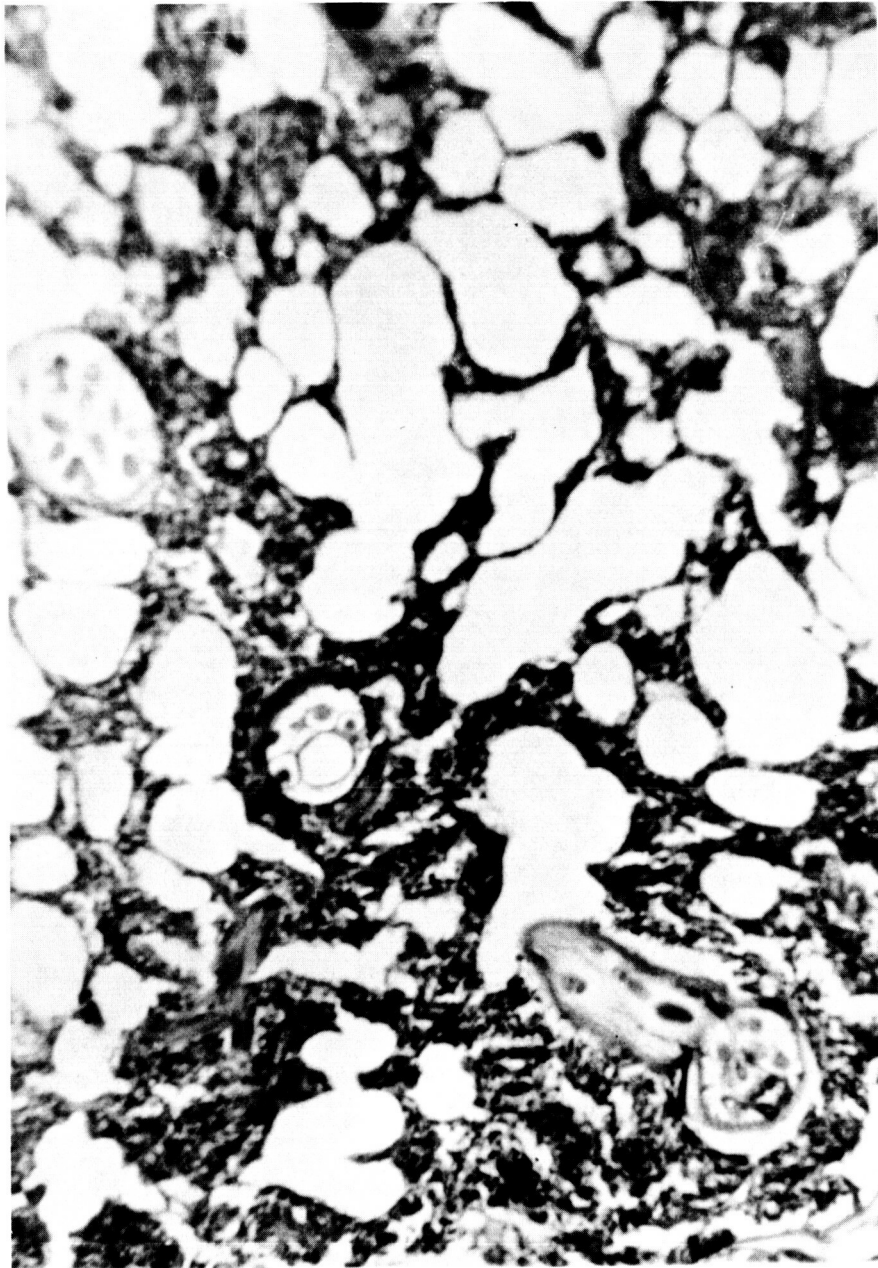


Figure 2

Lung of Squirrel Monkey N-8
Note multiple areas of parasitic infestation in lung parenchyma with
related inflammatory cell response in the adjacent stroma.
H and E. X 100

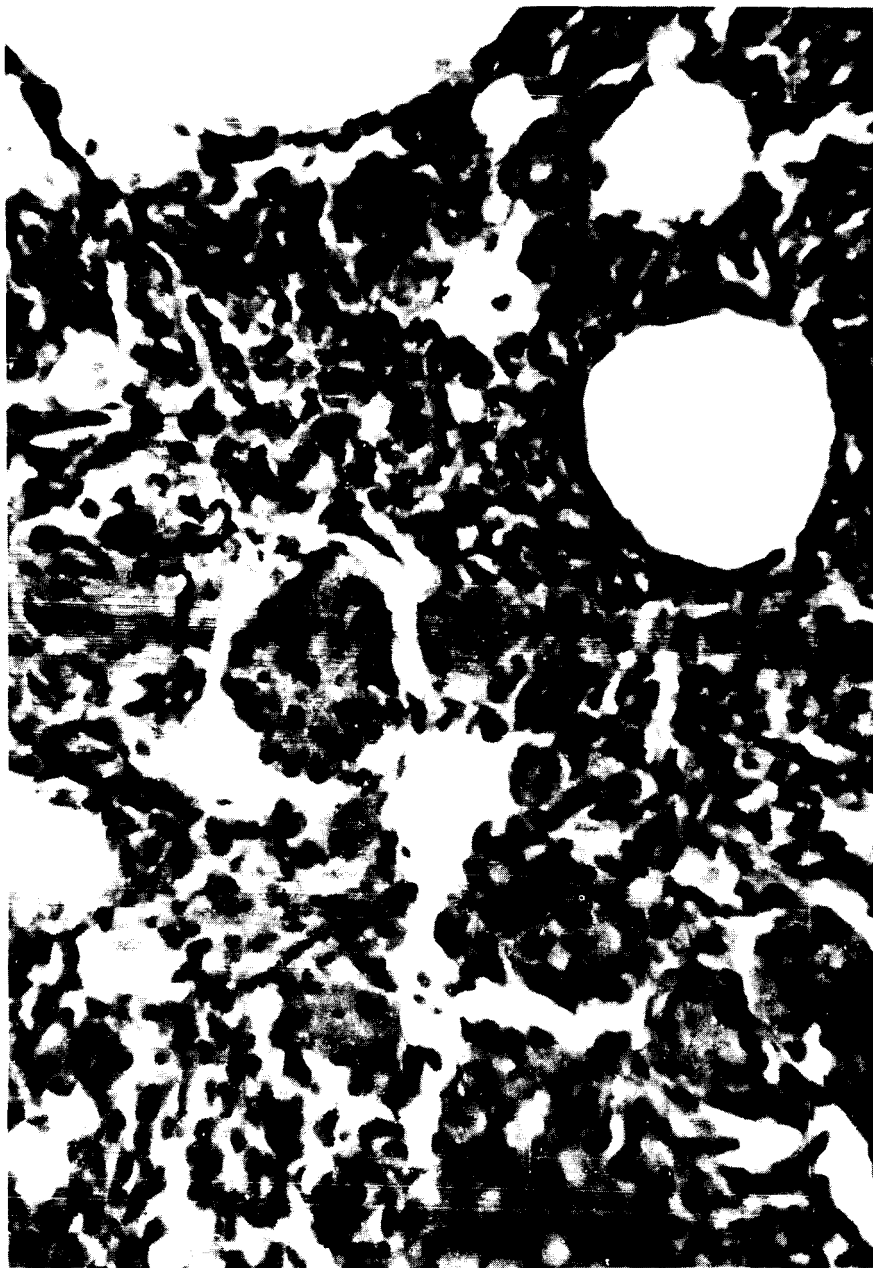


Figure 3

Lung of Squirrel Monkey N-8
Note areas of chronic inflammatory cells and giant cell formation.
H and E. X 450

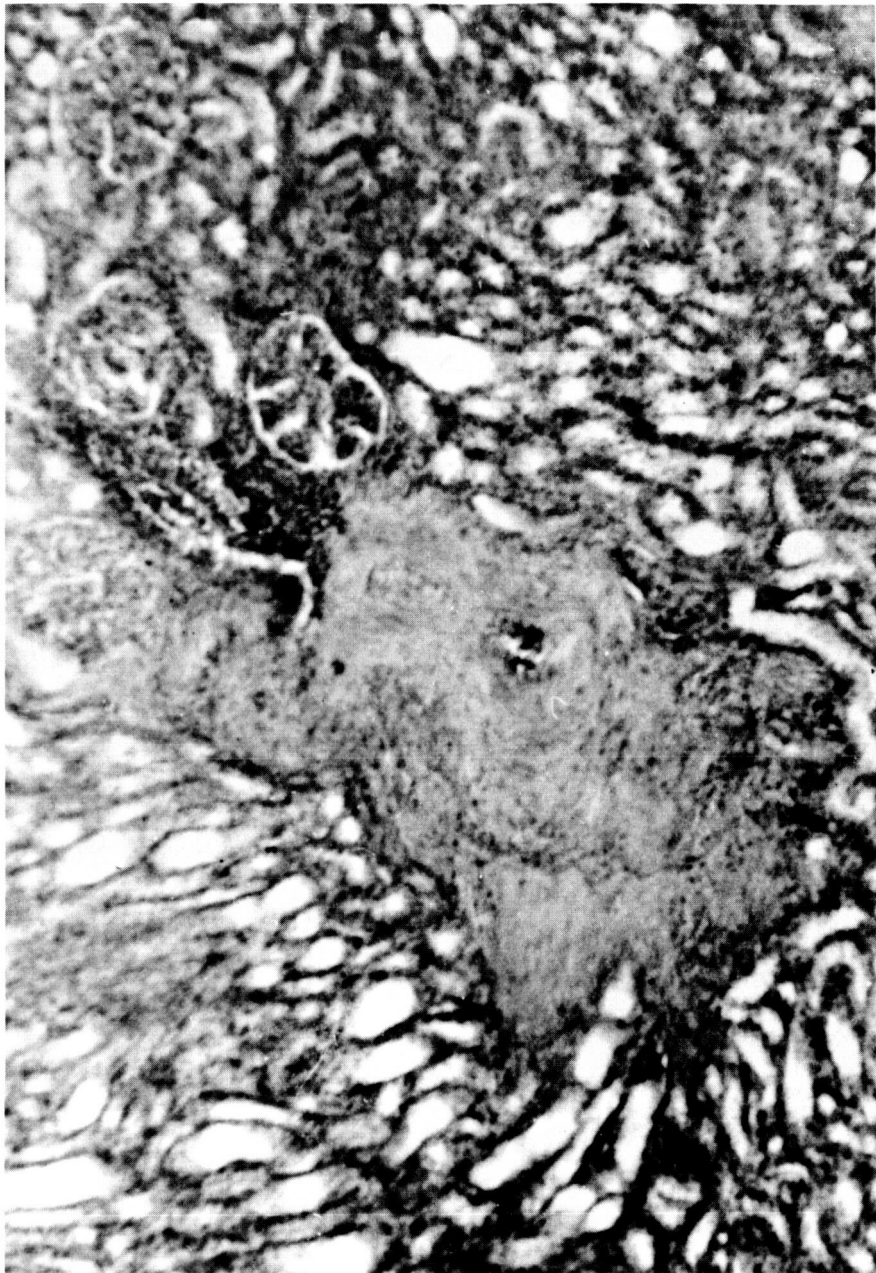


Figure 4

Kidney of Squirrel Monkey N-8
Large area of fibrosis with destruction of adjacent glomeruli and tubules.
H and E. X 100

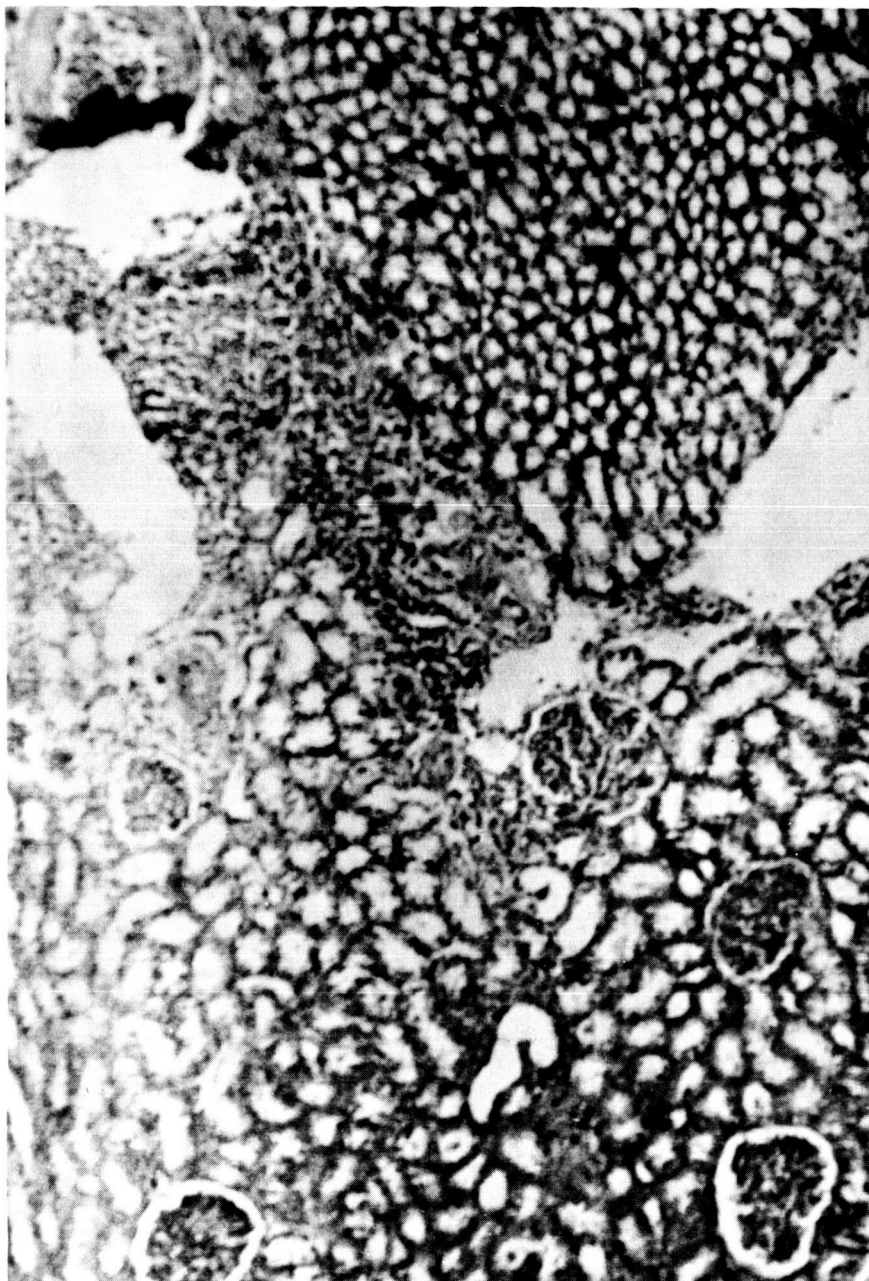


Figure 5

Kidney of Squirrel Monkey N-1
Note areas of acute inflammatory cells invading both cortex and medulla.
H and E. X 100

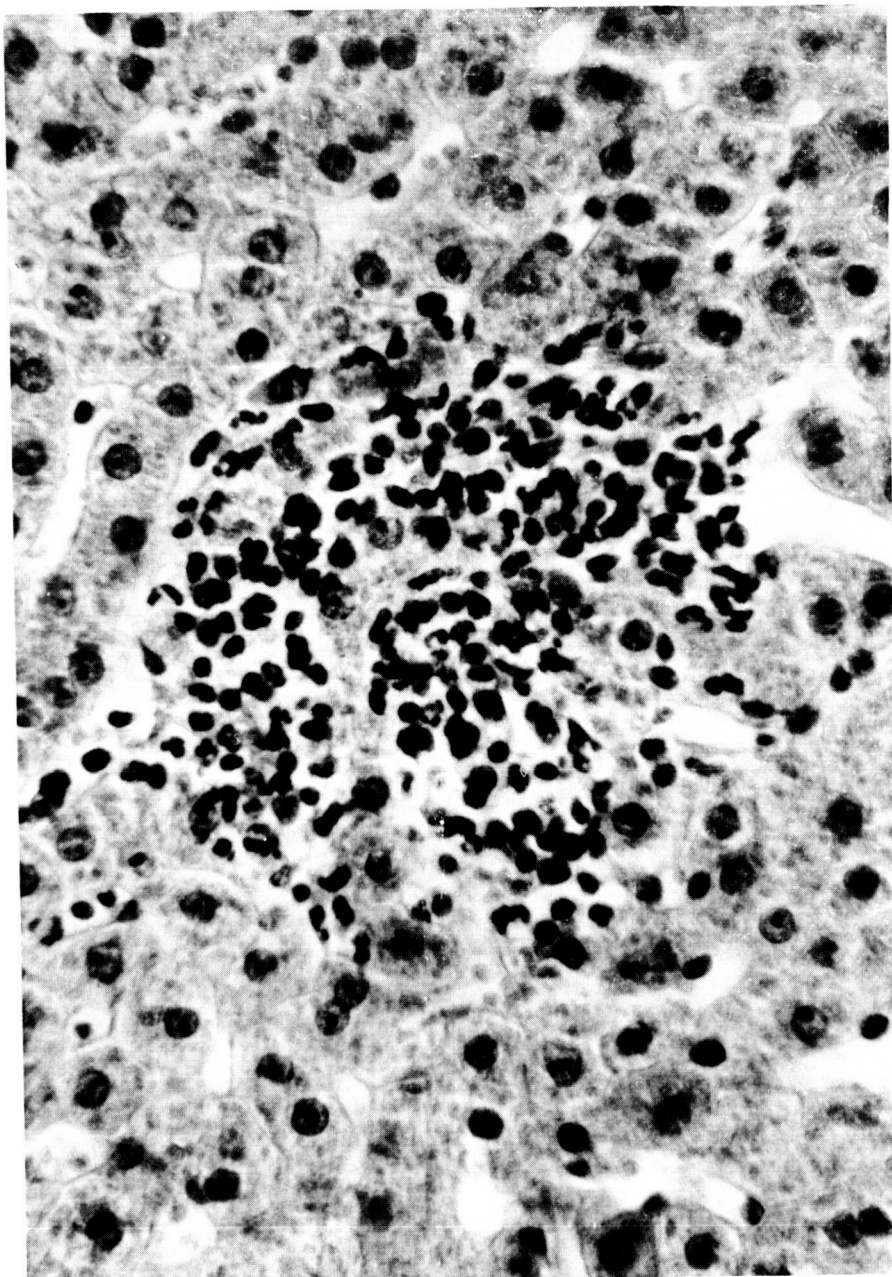


Figure 6

Liver of Squirrel Monkey N-5
Well-defined foci of leucocytic infiltration. Note essentially
normal adjacent parenchymal tissue.
H and E. X 450

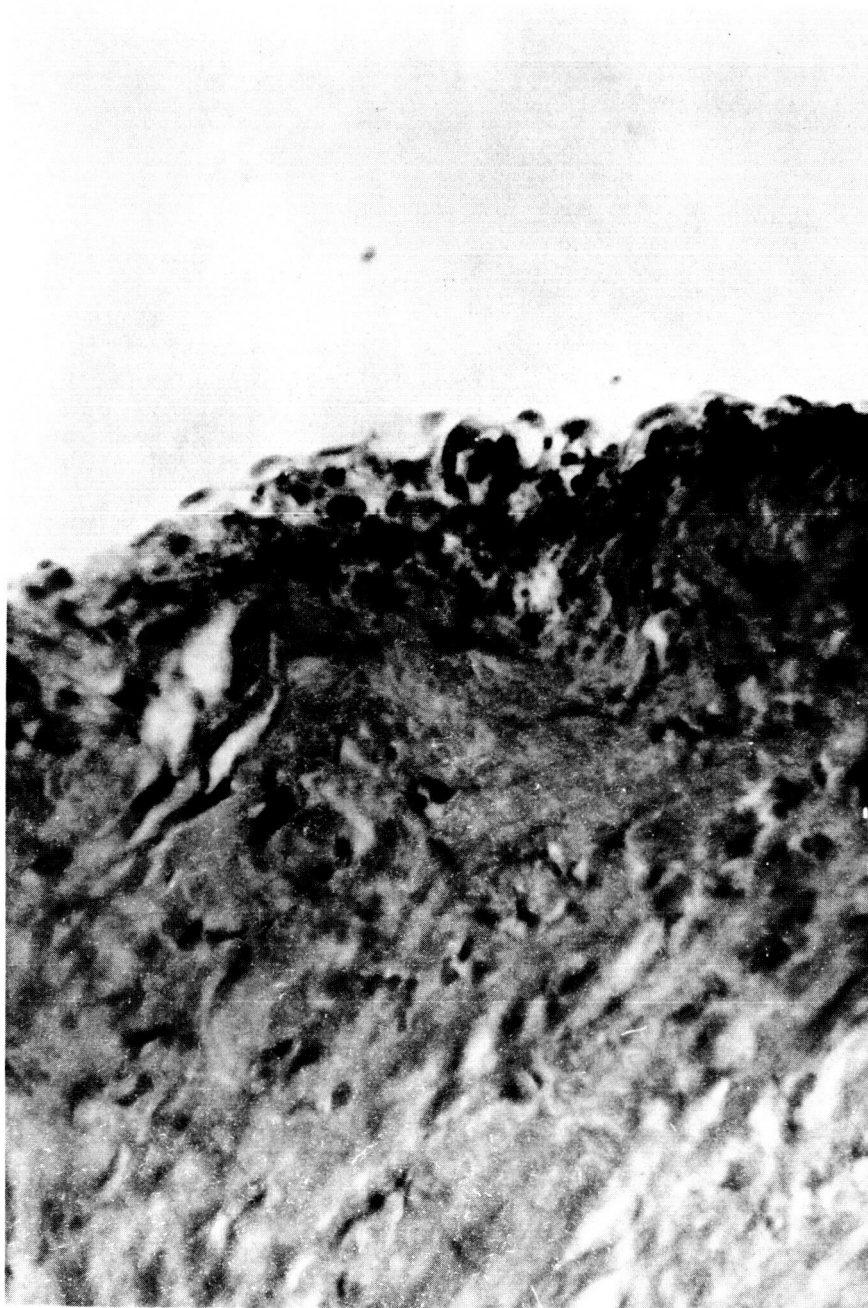


Figure 7

Ascending Aorta of Squirrel Monkey N-8
Note proliferation of endothelium, with fibrin deposition in the adjacent intima.
H and E. X 450

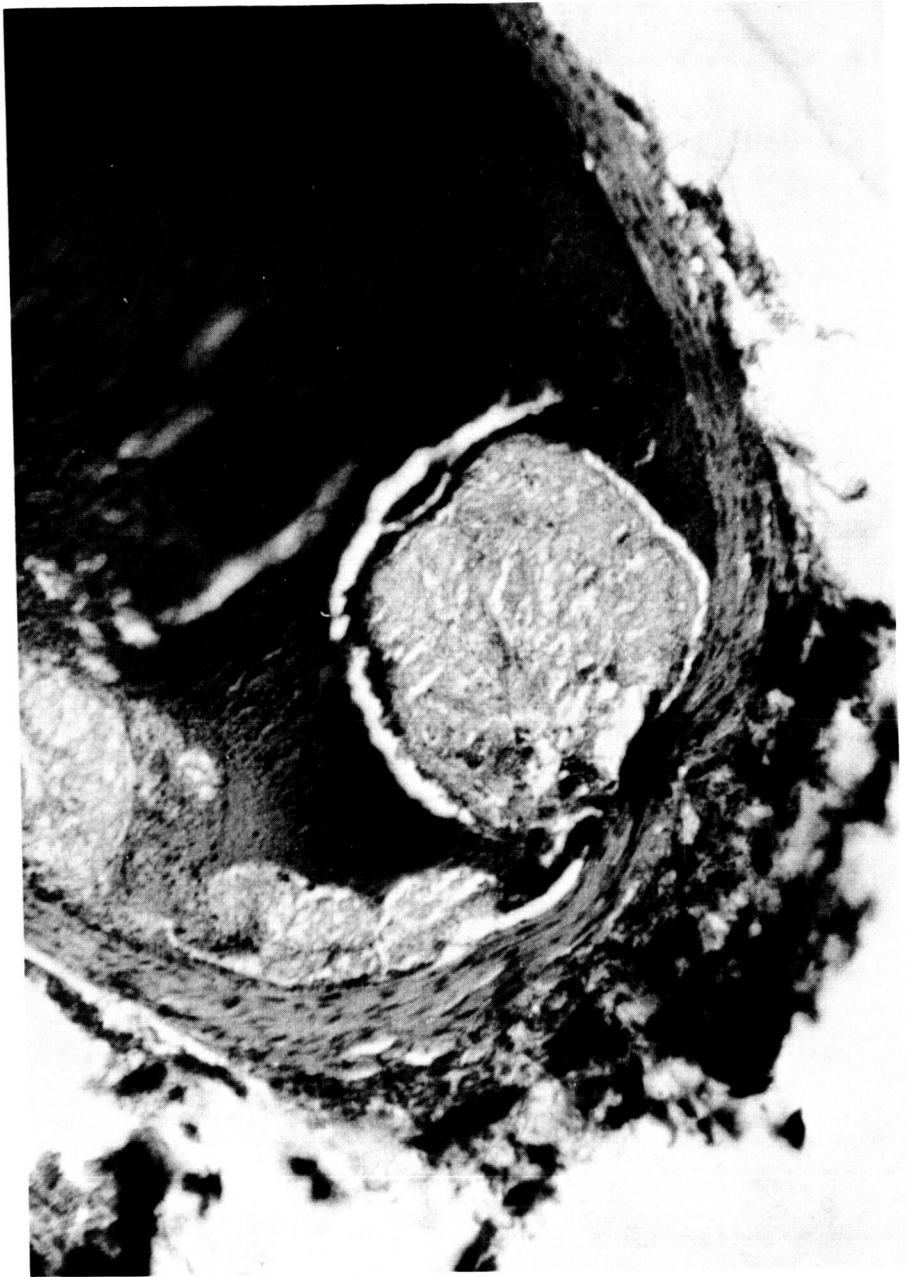


Figure 8

Renal Vein of Squirrel Monkey N-5
Note organized thrombus.
H and E. X 100



Figure 9

Lung of Squirrel Monkey P-4

Note island of nondescript matrix with remnants of alveolar cells
and reticulum, fibrin, and acute inflammatory cells.

H and E. X 100



Figure 10

Colon of Squirrel Monkey P-4
Note granuloma invading submucosa and mucosa with
chronic inflammatory cells in lamina propria.
H and E. X 100